Steps for Problem 6

**Module One Ligation of Vector:**

1. Get three 1.5ml microtest tubes and label 1-3
2. To tube 1 (stock ligation reaction mixture)
   1. add 40ul of qualified water
   2. add 20ul of vector and kanr fragment
   3. mix by tapping or vortexing
3. To tube 2
   1. Add 20ul from tube 1
   2. Add 5ul of 10x gel load
   3. Mix by tapping
4. Vortex T4 DNA ligase Reaction Tube (C)
5. To the T4 DNA ligase Reaction Tube add 40ul from tube 1
6. Incubate this for 5 minutes at room temperature
7. Pipette the T4 DNA ligase reaction tube up and down
8. Pulse in a centrifuge
9. Incubate at room temperature for 1 hour or at 16⁰C water bath for 30 minutes
10. Vortex or tap periodically during incubation
11. In tube three
    1. Add 20ul of the T4 DNA ligase reaction tube
    2. Add 5ul of 10x gel load
12. Tube 2 and 3 will be used for electrophoresis or stored frozen for later use.

**Storage point frozen**

Module 2: Transformation and Selection

1. Get three 1.5ml microcentrifuge tubes
2. Get one 1.5ml microcentrifuge tube label C for Control
   1. Add 500ul (0.5ml) of ice cold 0.05MCaCl2
   2. Place on ice
   3. Overload your inoculating loop with well-isolated colonies
   4. Twirl the loop in the tube to suspend the cells
   5. Vortex
3. Get one 1.5ml microcentrifuge tube label L for ligation
   1. Add 250ul of the cells from step one
   2. Place on ice
4. Get one 1.5ml microcentrifuge tube label DLR DNA
   1. Add 5ul from T4 DNA Ligase Reaction Tube (module 1)
   2. Add 45ul of qualified water
   3. Vortex
5. Add 10ul of DLR DNA to tube L, vortex, put on ice
6. Add 5ul of supercoiled plasmid DNA to tube C, vortex, put on ice
7. Incubate tube L and C on ice for 15 minutes
8. Place tubes L and C in 42⁰C waterbath for 90 seconds
9. Return tube to ice bath for 2 minutes
10. Add 250ul of Recovery broth to each tube, mix
11. Incubate tube for 30 minutes in a 37⁰C waterbath
12. Spin tube for 5 minutes
13. Discard 400ul of supernatant and suspend the pellet in remaining liquid
14. Obtain two Kanamycin plates
15. Label one control and one ligation
16. Pipet 100ul of tube C to the control plate
17. Pipet 100ul of tube L to the Ligation plate
18. Spread the liquid using a sterile loop, turn the plate 90⁰ and spread again
19. Let plates set upright to allow liquid to absorb
20. Incubate at 37⁰C overnight

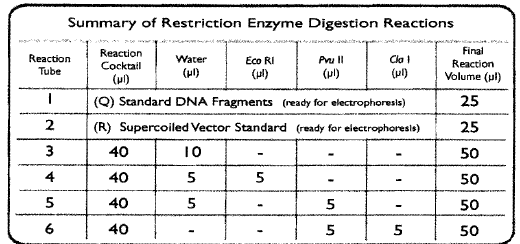
Module 4: Extraction of Recombinant plasmids from Kanr transformants

1. Obtain an 1.5ml microcentrifuge tube
   1. Add 200ul of Resuspension Buffer
   2. Take 10-15 colonies from the Ligation plate and suspend in tube
   3. Add 5ul of RNAse
   4. Incubate for 5 minutes at room temperature
   5. Add 350ul of Lysis buffer, cap and invert 4 to 5 times (DO NOT VORTEX)
   6. Add 200ul of Potassium Acetate, cap and invert 4 to 5 times
   7. Place tube on ice for 5 minutes
   8. Centrifuge at full speed for 5 minutes
   9. Transfer supernatant into a new 1.5ml tube, **measure this volume**
2. Add 600ul of Isopropanol for every 1ml of supernatant
3. Mix by inverting 4 to 6 times
4. Incubate at room temperature for 10 minutes
5. Centrifuge at full speed for 5 minutes
6. Remove supernatant
7. Add 350ul of Ethanol
8. Centrifuge at full speed for 3 minutes
9. Remove supernatant
10. Add 50ul of 1x TE buffer, cap and vortex
11. Pulse sample once in centrifuge (high rpm in a short amount of time)

**Storage point frozen**

Module 5: Restriction Enzyme Analysis

1. Obtain a 1.5ml tube
   1. Add 150ul of qualified water
   2. Add 25ul of restriction reaction buffer
   3. Add 25ul of suspended recombinant plasmid (from module 4)
2. Following the chart below put content in the appropriate micro tubes



1. Incubate tubes 3-6 at 37⁰C for 1 hour
2. Add 5ul of 10x loading solution to tube 3-6

**Storage point frozen**

1. Incubate the samples in a 65⁰C waterbath for 2 minutes, if frozen
2. Perform electrophoresis for analysis